Computerized Densitometry and Color Coding of [14C] Deoxyglucose Autoradiographs

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A computerized image processing system has been developed for quantitative analyses of autoradiographs obtained with the [¹⁴C]deoxyglucose method. The system is composed of standard, commercially available components and includes a scanning microdensitometer, computer, image memory and display system, and monochrome and color monitors. The associated computer programs are written in PASCAL. Autoradiographs are automatically scanned, and the optical density of each spot is digitized at a maximum resolution of 65,536 readings per 6.4 × 6.4 mm area and stored in memory. Images can be reconstructed from the data in memory, displayed on the monitors, and utilized for microdensitometric analyses or manipulated for image enhancement, enlargement, or weighted averaging of selected regions. The digitized data can also be utilized to solve the operational equation of the [¹⁴C]deoxyglucose method, and color-coded images of autoradiographs can be reconstructed so that each color represents a narrow range of the rate of glucose utilization. By means of this system, it is possible to generate quantitative metabolic maps that display the distribution of actual rates of local glucose utilization throughout the entire central nervous system in regions as small as 100 µm or less.

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A recent useful application of quantitative autoradiography has been the radioactive deoxyglucose method for determination of local cerebral metabolic rate [19]. This method, which employs tracer amounts of 2-deoxy-D-[14C]glucose ([14C]DG) to trace glucose metabolism through the hexokinasecatalyzed phosphorylation step, makes possible quantitative determination of the rates of glucose utilization simultaneously in all the macroscopic structural and functional components of the central nervous system in laboratory animals in either normal conscious or altered physiological states. The quantification of local cerebral glucose utilization is based on a kinetic model that incorporates the known biochemical properties of 2-deoxyglucose in brain tissue, the kinetics of the exchange of 2-deoxyglucose and glucose between plasma and brain, and the kinetics of their phosphorylation by hexokinase. Localization is achieved by measurement of tissue concentrations of carbon 14 in specific regions of brain by a quantitative autoradiographic technique. The method has been applied to a variety of experimental conditions in the rat [4, 18, 19] and monkey [6, 8, 9], and studies are in progress to adapt it to the dog (Duffy T: unpublished data, 1979) and

cat (Magnes J, Kennedy C, Miyaoka M, et al: unpublished data, 1979).

The amount of information stored in autoradiographs of brain obtained with the [14C]DG method is immense. The method is presently capable of measuring glucose utilization in regions as small as 100 μm. This level of resolution and the great heterogeneity of cerebral metabolic activity throughout the brain are clearly visible in autoradiographs. Because of the enormous complexity, it has been impracticable to recapture all the information present in autoradiographs, combine it with the quantitative potential of the [14C]DG method, and reconstruct highly detailed, spatially precise, quantitatively correct metabolic maps demonstrating the actual rates of glucose utilization throughout the tissues of the brain. Because of the practical limits of conventional manual densitometry, it has been necessary to subdivide the brain arbitrarily into discrete regions of metabolic activity that correspond more or less to traditional neuroanatomically defined entities and to determine an average value of glucose utilization based on an arbitrary finite number of densitometric measurements in each of these regions. In view of the great heterogeneity of metabolic activity within even

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well-defined anatomical structures, an inordinate number of densitometric readings has often been necessary to avoid sampling errors and to obtain a reliable estimate of the properly weighted mean value for metabolic rate within each structure. Data so obtained have generally been presented in large tables consisting of long but limited lists of named structures and their corresponding rates of glucose utilization. Such presentation of data ignores or obscures much of the information present in the autoradiographs, e.g., information about structures not included in the lists or about heterogeneity within each of the structures.

The present report describes a system for computer-assisted image processing of autoradiographs that greatly expands the analytical scope of the [14C]DG method. This system permits quantitative presentation of local rates of glucose utilization in the pictorial form of autoradiographs. Through color coding, the autoradiographs are transformed into representations of actual rates of local glucose utilization, and the heterogeneity and complexity in the distribution of local metabolic activities are fully retained and visualized. Furthermore, metabolic maps from different autoradiographs and from different animals can be normalized through the assignment of designated colors to specific rates of glucose utilization, thus permitting direct visual but quantitative comparison of autoradiographs from different experiments. Computer programs have also been developed which permit use of this system for highresolution densitometry. It is presently possible to determine the rates of glucose utilization in structures as small as approximately 100 μ m in width, and greater resolution may be possible in the near future. Other programming features permit determination of the average rate of glucose utilization for the brain as a whole, properly weighted for the relative masses of all its component parts.

Methods

Basic Principles of the [14C]DG Method

Before the design and capabilities of the computerized system are discussed, a brief summary of the [14C]DG method will be presented. A detailed description of the theory and procedures of this method has recently been published [19].

The deoxyglucose method is based on accumulation of 2-deoxy-D-[14C]glucose-6-phosphate in tissue following an intravenous pulse of 2-deoxy-D-[14C]glucose. Deoxyglucose is transported across the blood-brain barrier by the same carrier that transports glucose, and it competes with glucose for hexokinase, the enzyme which phosphorylates both to their respective phosphates. The rate of deoxyglucose phosphorylation is quantitatively related to the rate of glucose phosphorylation, depending on their relative concentrations in the precursor pools and the kinetic prop-

erties of hexokinase with respect to the two substrates. In a steady state of glucose metabolism, the net rate of glucose phosphorylation equals the rate of glucose utilization. On the basis of these kinetic principles, an operational equation has been derived that expresses the rate of glucose utilization in terms of defined constants and measurable variables, e.g., the final tissue concentration of ¹⁴C, the time courses of the arterial plasma glucose and [¹⁴C]DG concentrations, the rate constants for the transport of [¹⁴C]DG across the blood-brain barrier and its phosphorylation, and a combination of constants, the so-called lumped constant, consisting of the ratios of the distribution volumes and the Michaelis-Menten kinetic constants of hexokinase for deoxyglucose and glucose [19].

The experimental period is initiated by an intravenous pulse of [14 C]DG. Timed arterial blood samples are withdrawn during the ensuing period, and the plasma is analyzed for [14 C]DG and glucose concentrations. After 45 minutes the animal is decapitated, and the brain is removed and frozen in Freon XII chilled to -60° C with liquid nitrogen.

Brain sections 20 μ m thick are prepared with a cryostat (American Optical Co, Buffalo, NY) maintained at -21° to -22° C. The brain sections are picked up on a cover glass, dried on a hot plate at 60° to 70° C, and then placed sequentially in an x-ray cassette with Kodak SB-5 x-ray film (Eastman Kodak Co, Rochester, NY). Exposure time is generally five to six days. Calibrated [14C]methyl methacrylate standards are included with the brain sections during autoradiographic exposure; these are used to obtain a calibration curve for each film for conversion from optical density to tissue ¹⁴C concentration.

The Computer-assisted Image Processing System

The computerized system developed to process the autoradiographic data includes a rotating-drum scanning densitometer, a computer, a disk storage system, an image display system, monochrome and color monitors, joystick controls, a video terminal, and a line printer (Fig 1).

The rotating-drum scanning densitometer (Model P-1000, Optronics International, Chelmsford, MA) is used to convert the photometric data from the autoradiographs into digital form. The film containing the autoradiographic images of the brain sections and the 14C plastic standards is positioned on the scanner drum. Under computer control a digitizing scan can be performed on any area of the film from a maximum size of 23 \times 23 cm to a minimum of 6.4 \times 6.4 mm. Aperture sizes of 25, 50, and 100 μ m are possible for both the incident and collecting objectives. At its highest resolution, 40 lines per millimeter, the scanner permits access of 8,000 × 8,000 (i.e., 64,000,000) distinct data points per 20×20 cm of film area (1,600 data points/mm²). The currently employed image processing system, however, is capable of presenting simultaneously only a 256 × 256 array of data points, i.e., 65,536 points. If the area of the film to be scanned contains more than 256 × 256 potential readings, then readings are selected from the area at equal intervals to reduce the total number to the 65,536 permissible limit.

The scanner provides discrimination of 256 density levels (8 binary digits, or bits, per reading) over the optical

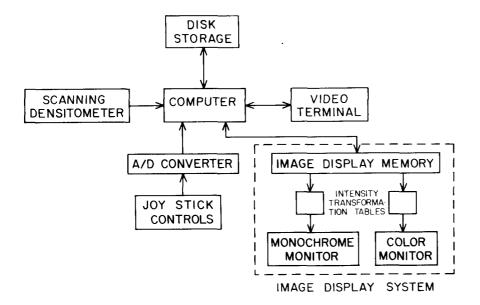


Fig 1. Components of the computer-assisted image processing system. The identities and sources of all components are specified in the text.

density ranges of 0 to 2 or 0 to 3. According to the manufacturer's specifications, density readings are reproducible to 2 parts in 256, and positional accuracy is rated at $\pm 2 \mu m$ per centimeter. The time required for a complete 256 × 256 scan ranges from 30 seconds to 2 minutes.

The 256 × 256 array of optical density readings is processed through a digital computer (Model PDP 11/34, Digital Equipment Corp, Maynard, MA) and is stored in the image memory of the image display system (Model ID-2000 Image-Display System, DeAnza Systems, San Jose, CA). The image display system includes a monochrome image transformation table which serves to convert the digital data stored in the image display memory into electrical signals that determine the intensity of the beam on the screen of the cathode ray tube of a monochrome monitor, and also a color intensity transformation table which controls the conversion of data into the combination of red, green, and blue signals necessary to create the appropriate color image on the face of the color monitor. The monitors are continually refreshed from the image display memory via the transformation tables at a frequency of 30 times per second, thus creating a 256 \times 256 element display on a Model 8025 high-resolution, 19 inch color monitor (Aydin Controls, Fort Washington, PA) and a Model BH high-resolution 13 inch monochrome monitor (Ball/Miratel, St. Paul, MN). The image display memory provides 12 bits for each of the 256×256 data points, the lower 8 containing the value for optical density, the upper 4 bits providing the opportunity for information coding, graphic overlays, area outlines, and other functions.

A random-access disk storage system (Model PM-DS/ 11B, 5.0 Mbyte Cartridge Disk System, Plessey Microsystems, Irvine, CA) permits storage and later recall of any 256 × 256 display. Approximately eight seconds is required for either operation.

Primary user-system interaction is accomplished through a video terminal (Model VT52 terminal, Digital Equipment Corp, Maynard MA). Two joysticks (i.e., control levers that serve as manual controls for bidimensional potentiometers; Model 525 x-y Potentiometer Joy Sticks, Measurement Systems, Norwalk, CT) and the requisite analog-to-digital (A/D) converter (Model AR11 Analog Real-time Subsystem, Digital Equipment Corp, Maynard, MA) permit additional user interaction with the image processing system. For example, the joysticks can be used to generate and adjust the size and position of a rectangle around a region of the display for more detailed analyses of the data within it.

Software developed to coordinate this system was written in PASCAL, a computer language which greatly facilitates the construction of large computer programs (OMSI PASCAL-1 compiler, Oregon Minicomputer Software, Portland, OR) [5, 7].

Results

Computer-assisted Image Enhancement

Image enhancement refers to manipulation of an image to present the observer with additional information which was less obvious in the preenhanced image. A number of image enhancement techniques have been reported [1, 2]. Four of these techniques—contrast stretching, intensity-window slicing, pseudocolor coding, and digital zoominghave been adapted for our purposes.

MONOCHROME IMAGE ENHANCEMENT. Contrast stretching and intensity-window slicing are methods for improving the appearance of monochrome images. These techniques affect the way in which the optical density readings stored in the image display memory are converted to shades of gray in the image presented on the monochrome CRT monitor. The "gamma" function for a monochrome image display

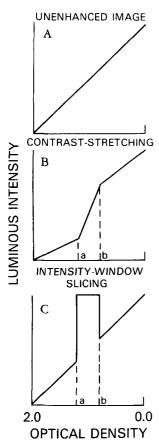


Fig 2. Methods to manipulate digitized image data for purposes of enhancement of monochromatic cathode ray tube (CRT) images. The graphs illustrate computer-generated relationships between image luminous intensity and optical density. (A) Normal relationship between image displayed on monochrome CRT and optical density data derived from original source (see Fig 3A). (B) Enhancement of contrast in the selected range of optical density (as, for example, between a and b in this illustration) (see Fig 3B). (C) Method of highlighting specific regions within a selected range of optical density (as, for example, between a and b in this illustration). Structures having optical densities within this range are displayed with high intensity on the face of the CRT (see Fig 3C).

system represents the relationship between the optical density readings which exist in the image display memory and the luminous intensities which are produced on the screen in correspondence with those numbers. A linear gamma function, such as that shown in Figure 2A, represents the matching of film optical density to monochrome image luminous intensity that most faithfully reproduces the image observed on the original autoradiograph.

Contrast stretching and intensity-window slicing involve the production of nonlinear gamma functions according to specific criteria. Again, the sole object of these techniques is to improve the image appearance in terms of human viewing.

A typical gamma function for a contrast-stretched

image is shown in Figure 2B. The range of optical densities between points a and b has been assigned a broad range of luminous intensities. This technique is valuable for enhancing an image that contains data over only a limited range of optical densities. Compare Figure 3A with its contrast-stretched counterpart in Figure 3B.

The gamma function for an image that has been intensity-window sliced (Fig 2C) indicates that a selected range of optical densities (between points a and b) will be presented in the image with substantially higher luminous intensities than optical densities above and below the limits of this range. The technique has proved valuable in accentuating edges and contours in autoradiographic images. Compare Figure 3A with its intensity-window sliced counterpart in Figure 3C. The intensity-window slicing technique has also proved useful in identifying threshold levels of optical density and glucose utilization for quantitative purposes; this topic is discussed in a later section.

PSEUDOCOLOR CODING. Pseudocolor coding is a technique for transforming a monochrome film image into an enhanced color image. In essence, this technique involves division of a broad range of optical densities into subranges of optical density that are each assigned a distinct color for presentation.

Pseudocolor transformation takes advantage of the human visual system's high sensitivity to color variation as compared with its more limited ability to resolve shades of gray. If employed merely to represent the optical densities in color, it serves mainly to beautify autoradiographs and adds relatively little of scientific value, though it may help to identify the position and extent of specific structures. The pseudocolor transformation can, however, be used to add a third dimension to the initially two-dimensional display by converting the qualitative monochromatic autoradiograph into a quantitative color-coded image. The computer program includes a routine to calculate a least-squares best-fitting cubic equation from the optical densities and concentration values of the ¹⁴C plastic standards. A cubic equation was empirically found to be the polynomial equation of lowest order which closely approximates the relationship between the 14C concentrations of the standards and their optical densities. This equation represents a calibration curve which is used to convert optical density for the autoradiograph into equivalent tissue ¹⁴C concentration. On keyboard command, the program utilizes this equation to construct and display, adjacent to the image of the brain section, a color scale that encompasses the full range of colors present in the image of the brain section and is numerically calibrated at the boundaries of each of the colors in

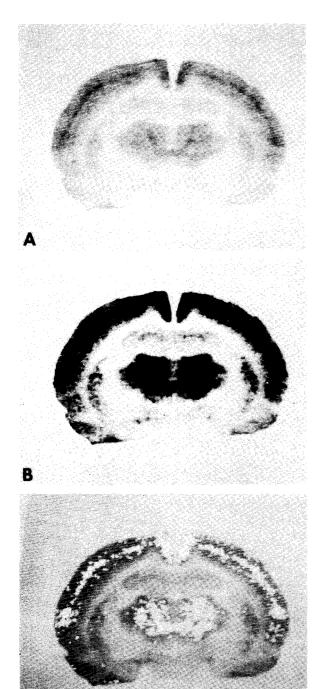


Fig 3. Reconstructed monochromatic images displayed on a CRT from a [14C]DG autoradiograph of a section of rat brain processed as described in Figure 2. The images in A, B, and C illustrate the results of image enhancement obtained by application of the methods illustrated in Figure 2A, B, and C, respectively.

units of local tissue ¹⁴C concentration. The program also contains the routines to solve the operational equation of the deoxyglucose method [19]. If the lumped constant, rate constants, and time courses of the arterial plasma [¹⁴C]DG and glucose concentrations are entered, then the program utilizes this equation as well, and constructs and displays a similar color scale but now calibrated in units of local cerebral glucose utilization. The autoradiographs are thus transformed into quantitative color-coded maps of the local rates of cerebral glucose utilization distributed anatomically throughout the brain section.

Because the scanning microdensitometer can discriminate and the DeAnza image display system can store 256 gray levels, it is possible to assign an individual color for presentation to each of the 256 density levels stored in the image display memory. In practice, however, color schemes in which the full range of values is divided among 12 to 20 colors have proved to be most useful in quantification of the deoxyglucose autoradiographs. One such 20-color scheme, a pseudospectral arrangement, is presented in Figures 4, 5, and 6. A 12-color arrangement suitable for individuals with red-green color blindness is shown in Figure 7.

The construction of each of these color schemes was based on three criteria:

- 1. The scheme should contain enough colors to allow for division of the full range of values in the autoradiographic data into sufficiently narrow ranges of glucose utilization to resolve relatively small differences in rate of glucose utilization.
- 2. The scheme should be "logical" (i.e., easily interpretable by the observer). The logical basis of the 20-color scheme in Figures 4, 5, and 6 is primarily wavelength (i.e., hue), with intensity (i.e., brightness) as a secondary factor. The basis of the 12-color scheme (Fig 7) is primarily intensity, with hue as a secondary factor.
- 3. The scheme should not contain discontinuities in hue or intensity that could lead to misinterpretation of results.

The division of the full range of optical densities in the autoradiograph into intervals represented by the assigned colors is arbitrary. Colors can be assigned to equal intervals of the full range. Alternatively, they can be assigned to intervals that increase in some continuous way, for example, geometrically or logarithmically. For example, proportionally narrower ranges of glucose utilization can be assigned to colors representing lower levels of glucose utilization, thus providing greater sensitivity in the lower ranges (see Figs 4, 5, and 6).

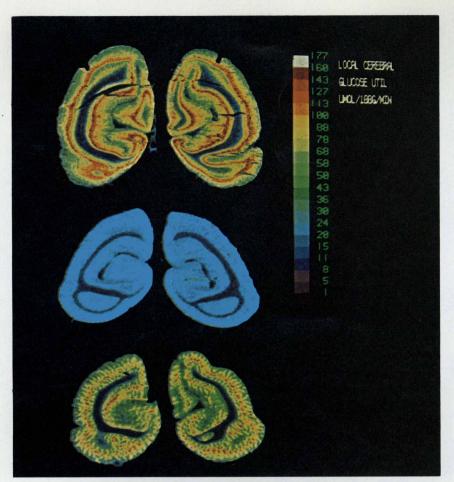


Fig 4. Pseudocolor reconstructions of [14C]DG autoradiographs of sections of the striate cortex from three rhesus monkeys: (top) both eyes open; (middle) both eyes patched; (bottom) right eye patched. The right hemisphere of the brain is on the right in these illustrations. (This illustration has been previously published in monochrome in Kennedy et al [8].)

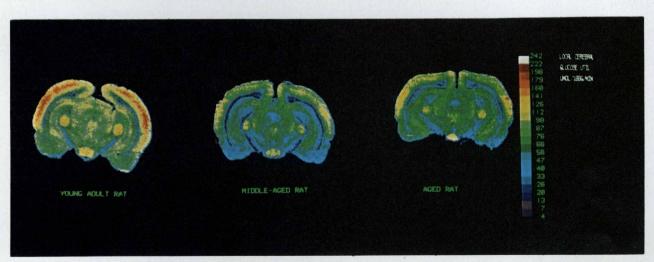


Fig 5. Pseudocolor reconstructions of [14C]DG autoradiographs of brain sections taken at the level of the medial geniculate body from rats representative of three age groups: (left) young adult, 4 to 6 months old; (middle) middle-aged, 14 to 16 months old; (right) aged, 26 to 36 months old. Note the generalized reductions in glucose utilization throughout the brain with age and reductions in the effective size and rate of glucose utilization in layer IV of the parietal and auditory cortices. (Illustration taken from unpublished data of C. B. Smith.)

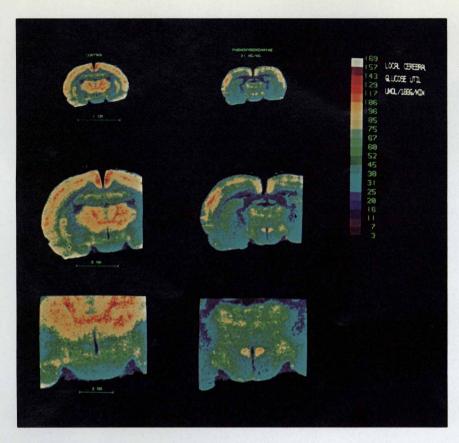


Fig 6. Pseudocolor reconstructions of [14C]DG autoradiographs from a normal conscious rat (left column) and a rat treated with phenoxybenzamine, 21 mg/kg (right column), taken at the level of the paraventricular hypothalamic nucleus: (top) scans of full sections with resolution of 100 data points/mm2: (middle) scans at increased resolution of 400 data points/mm2; (bottom) scans at highest level of resolution, 1,600 data points/ mm2. Note the prominence and detail observed in structures as small as the paraventricular nucleus, which is metabolically activated in the phenoxybenzamine-treated animals by the associated hypertension. (Illustration taken from unpublished data of H. E. Savaki.)

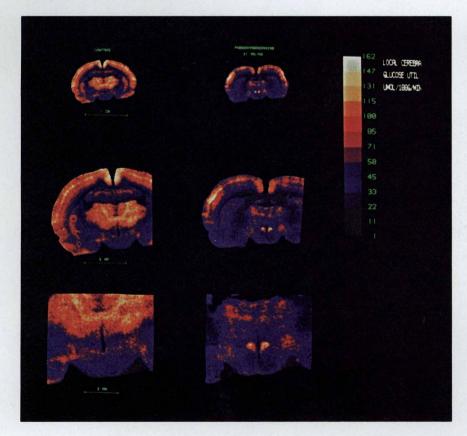
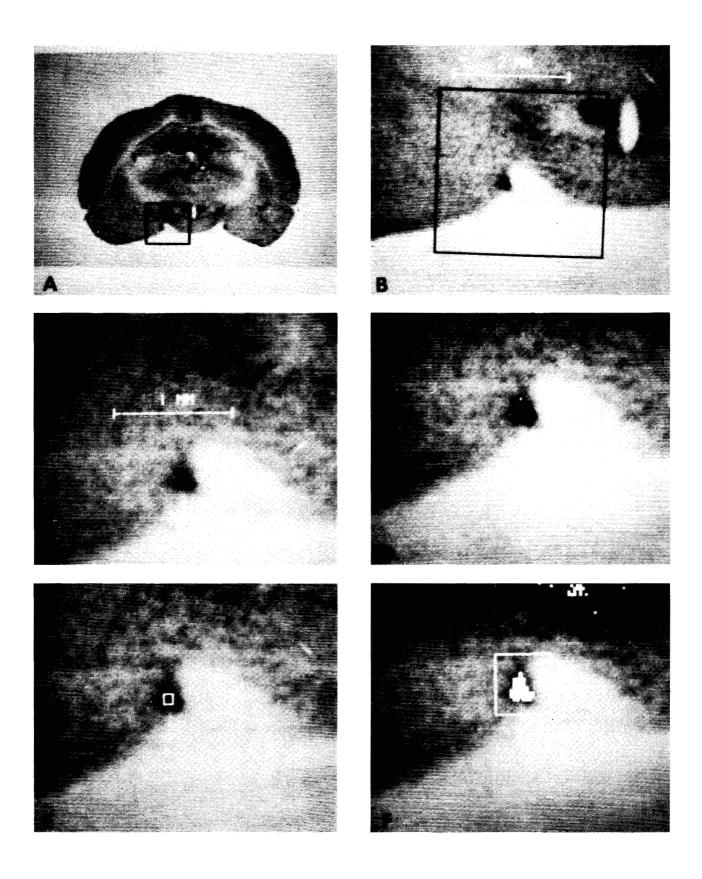


Fig 7. Alternative schema of pseudocolor coding. The autoradiographs reconstructed are the same as those in Figure 6, but the color scheme is based primarily on intensity, with hue as a secondary factor (see text).



DIGITAL ZOOMING AND RESCANNING AT IN-CREASED RESOLUTION. Digital zooming involves magnification of an area of interest in the image displayed on the monochrome and color monitors. Any 128×128 area of the 256 \times 256 image display memory can be expanded to fill the entire 256×256 memory array, each of the 128×128 elements in the original data becoming four elements in the new 256 × 256 image. This magnification process can be repeated as many times as desired. The zooming process is most often utilized to magnify an area already scanned at highest resolution (1,600 points/mm²) to bring out even more detail. Figure 8B was produced by rescanning at highest resolution a 6.4×6.4 mm area (the area bordered by the rectangle) of the section represented in Figure 8A. Figure 8C was produced by digital zooming of a 3.2×3.2 mm area (bordered by rectangle) of Figure 8B. The distinction between rescanning and digital zooming is important. Rescanning involves the collection of 256×256 new data points from a smaller area of the film with an associated increase in resolution. Digital zooming involves magnification of a portion of existing data stored in the image display memory and therefore results in no further increase in resolution.

Computer-assisted High-Resolution Densitometric Analysis

The capability of the image processing system to scan and store 256 × 256 individual readings from an area as small as 6.4×6.4 mm, and subsequently to display these data as a 20 × 20 cm image on the monochrome and color monitors, provides resolution and

Fig 8. Examples of features of the image processing system useful for microdensitometric analysis of [14C]DG autoradiographs. (A) Scan (100 data points/mm²) of a full section from the brain of a phenoxybenzamine-treated (42 mg/kg) rat taken at the level of the supraoptic nucleus. Computergenerated rectangle encompasses area of interest, including supraoptic nucleus, for rescanning at higher resolution. (B) Scan of region of autoradiograph containing the supraoptic nucleus at highest resolution (1,600 data points/mm²). Computer-generated rectangle encompasses region selected for image magnification. (C) Image magnification of area in the rectangle in B obtained by 2× zooming. (D) Operatorcontrolled computer-generated outline of area of interest, i.e., supraoptic nucleus, for computation of average optical density, 14C concentration, and glucose utilization within the designated area (see text). (E) Operator-controlled computergenerated rectangle for designation of area of interest selected for quantitative microdensitometric analysis (see text). (F) Combination of rectangular outlining and intensity-window slicing for defining regions in which average optical density, ¹⁴C concentration, and rate of glucose utilization are computed (see text).

quantification of brain structures at least as small as 100 μ m in width.

The precision of the measurement of optical density is primarily dependent on the character of the film (e.g., grain size and uniformity of emulsion) and the settings of apertures on the scanner. Analyses of replicate measurements of optical densities in autoradiographic images of the [14C]methyl methacrylate standards, uniform sources of approximately 10 to 20 mm², yielded coefficients of variation of $\pm 12\%$ for Kodak SB-5 x-ray film and ±8% for the finer grained Kodak MR-1 mammography film when scanned with apertures of 25 μ m, and coefficients of variation of $\pm 6\%$ and $\pm 5\%$, respectively, when scanned with apertures of 100 \(mu\)m. These values for the coefficients of variation were determined for images with an optical density of approximately 1.0. The signal-to-noise ratio of the instrument is also a factor, however, and an inverse relationship therefore exists between the coefficient of variation and the level of optical density being measured.

A factor that influences the accuracy of densitometric measurements is the so-called halo effect associated with ¹⁴C film autoradiography of tissue sections 20 µm thick. The influence of this factor has been evaluated by analysis of autoradiographs of sections of a rat brain that was itself unlabeled but contained a plug of uniformly labeled brain tissue inserted into it before sectioning. The results indicate that the halo effect diminishes semilogarithmically with distance from the true border, with a halfdistance of 50 (SD ± 10) μ m. Mainly for this reason, we doubt that the quantitative resolution of the method is finer than 100 μ m.

The optical density data resident in the image display memory can be utilized to compute the average optical density for any selected portion of the image. If the appropriate experimental constants have been entered, the data can be converted to mean tissue radioactive tracer concentration (in nCi/gm tissue) or to mean cerebral glucose utilization (in µmol/100 gm/min) for the selected portion of the image. The mean density, concentration, or rate of glucose utilization for a particular brain structure can be determined by any one of the three following procedures:

- 1. A selected portion of the image display corresponding to a given cerebral structure is surrounded by a visible outline constructed under joystick control (Fig 8D). The mean value within the outlined area is then automatically computed and is displayed numerically, along with the number and standard deviation, for the number of elements within the outline that were averaged.
- 2. A series of small rectangular areas are measured and collected from a selected structure and then

averaged by the computer (Fig 8E). The size of the rectangle and the number of readings collected are at the operator's discretion.

3. The rectangular outlining and intensity-window slicing features have been combined to produce the third alternative procedure. A selected region of the display is outlined by a rectangle generated and positioned under joystick control. A particular range or window is specified for the values of optical density, radioactive tracer concentration, or glucose utilization to be analyzed. The upper and lower limits of the window are under the operator's control. An average is then computed for all the individual readings within the outlined area that fall within the specified range. The readings which fall within that range are continuously highlighted on the monochrome monitor by means of the intensity window-slicing technique previously discussed (Fig 8F).

To illustrate the capability of the system for quantitative densitometry, consider that the density, tissue concentration, or rate of glucose utilization in a 1 \times 1 mm area on the autoradiograph can be computed as the mean of 1,600 distinct readings, 1 for each 25 \times 25 μ m region within that area. This capability allows determination of true weighted averages for each structure and resolves the problem encountered with manual densitometry of achieving adequate sampling of readings from any given structure.

Because the scanner takes readings at equally spaced intervals, the number of readings sampled from each individual structure is directly proportional to the area of its representation on the autoradiograph. The mean of all the readings over an area is therefore weighted for the sizes of all the structures represented in that area. This capability for weighted averaging, together with the speed with which the computer can access data, permits rapid determination of the weighted mean glucose utilization for an entire brain section as well as for individual regions within the section. From mean values of glucose utilization and the number of data points contributing to each mean collected from serial sections throughout the brain, it is also possible to compute from the tens of thousands of data points the average rate of glucose utilization in the brain as a whole, properly weighted for the masses of its component parts.

Discussion

Computer-assisted image processing was developed in the early 1960s during the National Aeronautics and Space Administration's early unmanned space program. Expansion of the technology of image processing was subsequently stimulated by requirements of the intelligence community for analysis of pictorial data [11]. Continued advances in computer technology have only recently reduced the barriers of cost and processing time sufficiently to encourage proliferation of image processing systems within the scientific community at large. A number of reports on the design and programming of image processing systems have been published and are helpful for guidance in the development of image processing techniques for specific uses [1–3, 11–13, 15].

The application of image processing techniques to the [14C]DG method evolved from a need to combine more effectively the method's capabilities of quantitatively determining and anatomically localizing the rates of glucose utilization throughout the central nervous system. Autoradiography is an integral part of the method, and the autoradiographs obtained with it often present a striking illustration of the pattern of distribution of metabolic activities throughout the brain. Their interpretation, however, is severely limited. First of all, they are not fully quantitative; the shades of gray in a [14C]DG autoradiograph depict only the relative rates of glucose utilization in the structural components of the brain. Autoradiographs from different experiments cannot therefore be directly compared, except to search for differences in the distribution of optical densities among the various cerebral structures. It is then often uncertain whether areas of apparent increase in relative density indicate heightened glucose utilization in that area or decreased utilization in other regions. Such questions can be resolved only by full quantification. Quantification has thus far required manual densitometry, which involves the measurement of optical densities in selected structures of the brain.

Experience with the method has uncovered a number of problems. Enormous numbers of readings must be made to ensure that sampling of each structure is adequate to provide true representative values for each region. It has been found, however, that anatomical structures are usually far from homogeneous. If this heterogeneity is ignored by sampling the entire structure, potentially valuable information is lost. If the structures are further subdivided according to heterogeneity, then enormous, tedious, and cumbersome tables of data are generated. Finally, structures of interest are often not visualized, and therefore not measured, because of their small size or the limited ability of the human visual system to discriminate shades of gray.

The computer-assisted image processing system described in this report largely resolves these problems. It scans and digitizes autoradiographs and reconstructs them on a color monitor in pseudocolor, which adds to the spatial distribution a third dimension representing the actual rate of glucose utiliza-

tion. The rate of glucose utilization can then be localized visually directly in each locus of the brain with a spatial resolution of at least 100 μm. Present equipment is capable of even finer spatial resolution. It can scan with an aperture of 25 μm and can accumulate and store 256×256 data points in a $6.4 \times$ 6.4 mm area. The 100 µm limit on resolution is therefore not in the image processing system; it is a result of the grain size of the film and the halo effect associated with 14C autoradiography. Similarly, use of color coding enhances the resolution of differences in rates of glucose utilization because of the ability of the human visual system to discriminate colors and tints. Reconstructed color-coded autoradiographs greatly increase perception of the marked heterogeneity of metabolic activities throughout the brain.

These color-coded metabolic maps of the brain are comparable to the maps of blood flow used by Lassen, Ingvar, and Skinhøj [10] to visualize rates of blood flow in the human cerebral cortex. Although their technique does not require the use of image processing, rates of blood flow computed from changes in counting rates measured with their batteries of fixed radiation detectors are coded into color and displayed spatially on a computer-constructed outline of the human cerebral cortex.

The benefits of image processing are not confined to the advantages of color coding. At the very least it provides rapid, semiautomated, comprehensive microdensitometry. Contrast enhancement makes it possible to visualize structures not obvious in autoradiographs because of insufficient differences in optical density between the structure and surrounding regions. Image enhancement by digital zooming or rescanning of smaller regions makes it possible to visualize microheterogeneity within even small structures, and measurements can be made in far smaller regions than is possible with manual densitometry. The capability for weighted averaging of local cerebral glucose utilization is also advantageous. Although the deoxyglucose method was designed specifically for measurement of glucose utilization in discrete regions of the brain, there are occasions when it is desirable to know also the average glucose utilization of the brain as a whole. It has hitherto been impossible to determine overall cerebral glucose utilization from local rates measured with the deoxyglucose technique because the overall average must be properly weighted for the relative masses of the individual structures. The weighting factors are generally unknown. The properties of image processing are such that when an area is averaged, the weighting factors are automatically incorporated because the number of readings per structure is directly proportional to the size of the structure. For example, values of local cerebral glucose utilization in the

conscious monkey have recently been reported [9]. Preliminary studies in these monkeys by the computer-assisted weighted averaging technique thus far indicate a value of 33 \(\mu\text{mol}/100\) gm/min for average glucose utilization by the brain as a whole, a value very close to those obtained previously by global techniques in very lightly anesthetized monkeys [16] or normal conscious young men [17].

The image processing system used in the present study represents our initial effort, and a relatively economical one, to apply image processing techniques to the mapping of local cerebral glucose utilization. Equipment and technology exist, however, to expand greatly the scope of the technique. Scanning microdensitometers are available that can scan with greater resolution at higher speeds. There are image memory and display systems that allow storage and display of 512 × 512 data elements rather than the 256×256 used here, thereby producing finer detail and resolution. With computer systems of greater capacity and speed, it should be possible to develop programs that reconstruct maps of the distribution of metabolic activity three dimensionally in the entire brain from data obtained by scanning of serial sections of the entire brain. Efforts in this direction are presently in progress in this laboratory.

Recent developments in computerized emission tomography have provided the means to apply the 2-deoxyglucose method to humans. A positronemitting derivative of deoxyglucose, 2-[18F]fluoro-2-deoxy-D-glucose, has been synthesized and found to retain the necessary biochemical properties of 2-deoxyglucose [14]. The resolution thus far obtained in human studies is considerably below that which has been obtained with autoradiography [14], but it can be expected to improve as a result of technological advances. Theoretical limitations inherent in positron-emission tomography, however, preclude the achievement of resolution equal to that possible with autoradiography. The image processing techniques described in this report can readily be adapted to tomographic data and, when combined with the [18F]fluorodeoxyglucose procedure, make it possible to obtain quantitative metabolic maps of glucose utilization throughout the human brain.

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